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Menthol Prevents Liver Damage Induced by Paracetamol and CCl_4

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Abstract: Menthol, a terpenoid from *Mentha piperita* was investigated for its possible protective effect against paracetamol and CCl_4-induced hepatic damage. Paracetamol produced 100% mortality at the dose of 1 g kg^{-1} in mice while pre-treatment of animals with menthol (50 mg kg^{-1}) reduced the death rate to 40%. Oral administration of paracetamol (640 mg kg^{-1}) produced liver damage in rats as manifested by the rise in serum enzyme levels of alkaline phosphatase (ALP) and transaminase (AST and ALT). Pre-treatment of rats with menthol prevented the paracetamol-induced rise in serum enzymes. The hepatotoxic dose of CCl_4 (1.5 ml kg^{-1}; orally) also raised the serum ALP, AST and ALT levels. The same dose of menthol was able to prevent the CCl_4-induced rise in serum enzymes and prolongation in pentobarbital sleeping time. In conclusion menthol possesses hepatoprotective activity, demanding further scientific evaluations to validate its future role in hepato-biliary complications.

Key words: Menthol, essential oils, *Mentha piperita*, hepatoprotective, paracetamol, CCl_4

Introduction

Menthol is one of the monoterpenoids present as essential oil in *Mentha piperita* and other related plants (McConkey *et al.*, 2000). It exhibits multiple biological activities including carminative (Eccles, 1994), expectorant (Riechelmann *et al.*, 1997), antibacterial (Pattnaik *et al.*, 1997), antifungal (Pattnaik *et al.*, 1997), anti-inflammatory (Juergens *et al.*, 1998), anti-pruritus (Greco and Ende, 1992) antiasthmatic (Wright *et al.*, 1998), hypotensive (Futami, 1984b), cytotoxic (Russin *et al.*, 1989), local anaesthetic (Galeotti *et al.*, 2001), immunomodulator (Sidell *et al.*, 1991), anti-platelets (Murayama and Kumaroo, 1986), calcium channel blocking (Dierkes *et al.*, 1997) and insecticidal (Lee *et al.*, 2001) activities. Moreover, it is also used as a counter-irritant (Futami, 1984a) and as a pharmaceutical aid to increase skin permeation to facilitate the absorption of medicaments through intact skin (Kaplun-Frischoff and Touitou, 1997). In present investigation, we describe its hepatoprotective activity against paracetamol and CCl_4-induced liver damage.

Materials and Methods

The study was conducted at Department of Bological and Biomedical Sciences, The Aga Khan University, Karachi in July-August, 2000.

Animals: Swiss male mice (20-25 g) and male albino wistar rats (200-250 g) were obtained from the Animal House of The Aga Khan University. The animals were housed in plastic cages (47 x 34 x 18 cm^3), mice (10/cage) and rats (5/cage), lined with sawdust renewed every 48 hours, in air-conditioned quarters and had free access to tap water and food.

Pharmacological materials: Paracetamol, CCl_4, menthol, ketamine hydrochloride and methyl cellulose were obtained from Sigma Chemicals Company, St Louis, MO USA and olive oil (P. Sasso e Figili, Oneglia, Italy) was purchased from local market. Paracetamol and CCl_4 were suspended in 1% methyl cellulose (50 mg ml^{-1}) and olive oil (20% v/v) respectively.

Lethality study in mice: Preliminary experiments were performed on mice to estimate the protective effect of menthol against a lethal dose of paracetamol (1 g kg^{-1}). Animals were divided into two groups of ten animals each. One group was treated orally with menthol (50 mg kg^{-1}) followed after 1 h by oral administration of paracetamol. The second group served as control and received the same treatment except that normal saline (0.9% NaCl) was administered instead of menthol. The mortality was observed for 24 h post-administration of paracetamol.

Hepatoprotective study: Hepatic injury in rats was induced separately by paracetamol (640 mg kg^{-1}) as well as CCl_4 (1.5 ml kg^{-1}), administered orally, whereas control animals received an equal volume of respective vehicle (1% methyl cellulose or olive oil), as described previously.

Rats were divided into 3 groups of 10 animals each. Group 1 served as vehicle control and received normal saline (10 ml kg^{-1}) and vehicle (1% methyl cellulose; 13 ml kg^{-1}, orally). Group 2 was given 4 doses of normal saline at 12-h intervals and paracetamol was administered orally 1 h post-treatment of the last dose. Group 3 was treated similar to group 2, except that menthol (50 mg kg^{-1}, dissolved in 10 ml saline) was administered instead of saline. In a parallel study on 3 similar groups of rats (n = 10), normal saline (10 ml kg^{-1}) and vehicle (olive oil; 7.5 ml kg^{-1}) were administered orally to vehicle control group, whereas the remaining 2 groups were treated similarly to the study mentioned above except that paracetamol was replaced by CCl_4.

Animals were anaesthetized with ketamine (100 mg kg^{-1}, i.m.) 24 h after the last treatment and blood (3 ml) was collected by cardiac puncture using sterile disposable syringes. Serum was separated by centrifugation (3000 rpm for 15 min) and serum alkaline phosphatase (ALP), aspartate transaminase (AST) and alanine transaminase (ALT) were estimated on the same day spectrophotometrically using Merck diagnostic kits.

Modification of CCl_4 -induced prolongation in pentobarbital sleeping time: The effect of menthol on CCl_4-induced prolongation in pentobarbital sleeping time was studied in mice as described elsewhere (Montilla *et al.*, 1990; Gilani *et al.*, 1998) (Table 2).

The animals were divided into three groups of ten animals each. Group 1 animals received four doses of normal saline (10 ml kg^{-1}) orally at 12-h interval and vehicle (olive oil) was administered as bolus dose (7.5 ml kg^{-1}; orally) 1 h after the last dose of saline followed after 24 h by pentobarbital (75 mg kg^{-1}, i.p), while animals of group 2 were given the same treatment except vehicle was replaced by CCl_4 (1.5 ml kg^{-1}). Animals in group 3 were treated similar to group 2 except that menthol (50 mg kg^{-1}) was substituted for normal saline.

Statistical analysis: The results were expressed as mean " SEM and all statistical comparisons were made by means of the student's t-test and $P < 0.05$ was regarded as significant.

Results

Effect of menthol on paracetamol-induced lethality: Paracetamol at the dose of 1 g kg^{-1} killed all mice. In a group of animals pre-treated with menthol (50 mg kg^{-1}), the same dose of paracetamol killed only four out of ten resulting in 60% protection against the lethal effect of paracetamol (Table 1).

Table 1: Effect of Menthol on Paracetamol-induced Lethality in Mice

Groups	Treatments	Mortality (%age)
1.	Menthol + Paracetamol (50 mg kg^{-1} + 1 g kg^{-1})	40
2.	Saline + Paracetamol (10 ml kg^{-1} + 1 g kg^{-1})	100

Table 2: Effect of menthol on CCl_4-induced prolongation in pentobarbital sleeping time in mice

Groups	Treatments	Sleeping time (minutes)
1.	Saline + Vehicle + Pentobarbital (10 ml kg^{-1} + 7.5 ml kg^{-1} + 75 mg kg^{-1})	112 ± 17
2.	Saline + CCl_4 + Pentobarbital (10 ml kg^{-1} + 1.5 ml kg^{-1} + 75 mg kg^{-1})	211 ± 21*
3.	Menthol + CCl_4 + Pentobarbital (50 mg kg^{-1} + 1.5 ml kg^{-1} + 75 mg kg^{-1})	127 ± 19#

Table 3: Effect of menthol on paracetamol-induced rise in serum enzyme levels in rats

Groups	Treatments	ALP	AST	ALT
1.	Saline + Vehicle (10 ml kg^{-1} + 13 ml kg^{-1})	211± 24	103± 18	44± 08
2.	Saline + Paracetamol (10 ml kg^{-1} + 640 mg kg^{-1})	335± 31*	1252± 298**	596± 147**
3.	Menthol + Paracetamol (50 mg kg^{-1} + 640 mg kg^{-1})	226± 20#	169± 31##	63± 12##

Group 3 animals received four doses of menthol (50 mg kg^{-1}) at 12-h interval before paracetamol (640 mg kg^{-1}) administration.

Table 4: Effect of menthol on CCl_4-induced rise in serum enzyme levels in rats

Groups	Treatments	ALP	AST	ALT
1.	Saline + Vehicle (10 ml kg^{-1} + 7.5 ml kg^{-1})	203± 20	98± 13	49± 14
2.	Saline + CCl_4 (10 ml kg^{-1} + 1.5 ml kg^{-1})	315± 27*	705± 186*	451± 121*
3.	Menthol + CCl_4 (50 mg kg^{-1} + 1.5 ml kg^{-1})	214± 19##	139± 27#	77± 23#

Values shown are mean ± SEM of 10 determinations expressed as IU. Group 3 animals received four doses of menthol (50 mg kg^{-1}) at 12-h interval before CCl_4 (1.5 ml kg^{-1}) administration. * $P < 0.05$; ** $P < 0.01$; Compared to group 1. # $P < 0.05$; ## $P < 0.01$; Compared to group 2.

Effect of menthol on CCl_4-induced prolongation in pentobarbital sleep: The effect of menthol on CCl_4-induced prolongation of pentobarbital sleeping time was studied in mice. Pentobarbital at a dose of 75 mg kg^{-1}, i.p., caused sleep in mice of control group for a period of 112± 17 min (mean± SEM; n= 10). Whereas treatment of animals with CCl_4, prolonged the pentobarbital sleeping time to 211 ± 21 min, the value that is significantly higher ($P < 0.01$) than that of control (Table 2). However, prior treatment of animals with menthol (50 mg kg^{-1}) returned this CCl_4-induced prolongation of pentobarbital sleeping time to 127± 19 min, which is significantly lower than group 2 animals ($P < 0.05$) and close to the control sleeping time ($P < 0.05$).

Effect of menthol on paracetamol-induced hepatotoxicity: Control (saline + vehicle) serum values of ALP, AST and ALT in rats were found to be 211± 24, 103± 18 and 44± 08 IU respectively (Table 3), while toxic dose of paracetamol (640 mg kg^{-1}) raised significantly ($P < 0.05$) the respective serum enzyme values to 335± 31, 1252± 298 and 596± 147. Group 3 animals were pre-treated with menthol (50 mg kg^{-1}) to determine its effects on paracetamol-induced rise in serum enzymes. The serum values of enzymes in pre-treated group were found to be 226 ± 20 (ALP), 169± 31 (AST) and 63± 12 (ALT), which were significantly lower ($P < 0.05$) than the values of toxic control and similar to the control values ($P > 0.05$).

Effect of menthol on CCl_4-induced hepatotoxicity: The estimated values of serum alkaline phosphatase (ALP) and transaminase (AST and ALT) in control (saline + vehicle) group of rats were found to be 203± 20, 98± 13 and 49± 14 IU respectively (Table 4), which were raised significantly ($P < 0.01$) to the respective values of 315± 27, 705± 186 and 451± 121 after administration of a toxic dose of CCl_4 (1.5 ml kg^{-1}). However, pretreatment of animals with menthol (50 mg kg^{-1}) returned the serum ALP, AST and ALT values to 214± 19, 139± 27 and 77± 23 IU respectively, which were significantly lower ($P < 0.05$) than values of toxic control and were close to normal values ($P > 0.05$).

Discussion

Paracetamol and CCl_4-induced hepatic injuries are commonly used models for hepatoprotective drug screening (Plaa and Hewitt, 1982) and the extent of hepatic damage is assessed by the level of increased cytoplasmic enzymes (ALP, AST and ALT) in circulation (Sallie *et al.*, 1991). Menthol when administered prophylactically exhibited protection against paracetamol-induced lethality in mice suggesting hepatoprotective actions.

The treatment of mice with CCl_4 caused a damage to microsomal drug metabolizing enzymes in hepatocytes leading to a substantial decrease in hepatic drug metabolizing capacity, being reflected in prolongation of pentobarbital-induced sleeping time (Javatilaka *et al.*, 1990). Whereas, pretreatment of animals with menthol prevented the CCl_4-induced prolongation in pentobarbital-sleeping time, suggesting a protective effect of menthol against CCl_4-induced damage to hepatocytes.

Paracetamol is converted to a toxic reactive intermediate called N-acetyl-p-bezoquinone imine (NAPQI) following metabolism by a number of isozymes of cytochrome P-450 (CYPs), i.e., CYP 2E1 (Tanaka *et al.*, 2000), CYP 1A2 (Lehman, 2000), CYP 2A6 (Chen *et al.*, 1998), CYP 3A4 (Dong *et al.*, 2000) and CYP2D6 (Dong *et al.*, 2000), whereas CCl_4 is activated to halogenated free radicals (HFR) by CYP 2E1 (Jeong, 1999). The massive production of reactive species may lead to depletion of protective physiological moieties (glutathione and α-tocopherol, etc.), ensuing wide-spread propagation of the alkylation as well as per oxidation, causing damage to the macro molecules in vital biomembranes (Aldridge, 1981).

The inhibitors of CYPs are known to curtail the toxicity of paracetamol (Sumioka *et al.*, 1998) as well as CCl_4 (Mizuoka *et al.*, 1999). Menthol treatment was able to ameliorate the paracetamol and CCl_4-induced hepatocellular damage as evidenced by prevention of any increase in serum enzymes (ALP, AST and ALT) levels subsequent to toxin exposure and the reported inhibitory effect of menthol on CYPs (De-Oliveira *et al.*, 1999) might be the contributing factor towards the observed hepatoprotection.

Moreover, menthol is reported to exhibit calcium channel blocking activity (Dierkes *et al.*, 1997) and also tends to lower the intracellular Ca^{2+} by activation of Ca^{2+} sequestration mechanisms (Starling *et al.*, 1994). Calcium content in liver cells are liable to be increased during the process of experimental liver damage (Tsokos-Kuhn, 1989) and calcium channel blocking drugs, i.e. nifedipine and verpamil were found to inhibit the development of hepatic damage induced by different hepatotoxins including paracetamol and CCl_4 (Thibault *et al.*, 1991). Similarly, hepatoprotective activity of menthol against paracetamol and CCl_4-induced damage reported in this study can be attributed to its calcium channel blocking activity.

Inflammation plays a central role during drug-induced acute

hepatitis and products of arachidonic acid metabolism have been extensively involved in inflammatory processes (Perez-Alvarez *et al.*, 1993). Similarly, the reported anti-inflammatory (Greco and Ende, 1992) as well as inhibitory effect of menthol on lipoxygenase (Greco and Ende, 1992) and cyclooxygenase enzymes (Greco and Ende, 1992) might also be partly involved in the protective effect against paracetamol and CCl_4-induced hepatotoxicity observed in this study.

In conclusion menthol exhibited protection against paracetamol- and CCl_4-induced liver injuries as manifested by the reduction in toxins-mediated rise in serum enzymes in rats, protection against lethal dose of paracetamol in mice and prevention of CCl_4-induce increase in pentobarbital sleeping time possibly through multiple pathways.

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